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An investigation into the nature of non-voiding contractions resulting from detrusor hyperreflexia in neurogenic bladders following spinal cord injury

PRINCIPAL INVESTIGATOR:
Matthew O. Fraser, Ph.D.

CONTRACTING ORGANIZATION:
Institute for Medical Research
Durham, NC 27705

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Matthew O. Fraser

E-Mail: matthew.fraser@duke.edu

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Institute for Medical Research
VA Medical Center (151-IMR)
508 Fulton Street
Durham, NC 27705

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14. ABSTRACT

The purpose of this project is to determine the cause behind non-voiding contractions (NVC) of the bladder seen with filling following suprasacral spinal cord injury (SCI). The most significant findings over the course of the past year have been that NVC in chronic SCI are accompanied by intra-abdominal pressure increases resulting from abdominal wall contraction. The temporal relationship appears to be that of either the abdominal pressure rise beginning just prior to, or simultaneously with, the bladder pressure rise, These results, therefore, strongly suggesting that these abdominal wall-mediated abdominal pressure rises either cause NVC, or arise from the same mass reflex event as the NVC.

15. SUBJECT TERMS
Spinal cord injury, neurogenic bladder, detrusor overactivity, detrusor hyperreflexia, mass reflexes, non-voiding contractions

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INTRODUCTION:

Suprasacral spinal cord injury (SCI) is often occasioned by numerous, rhythmic high pressure non-voiding contractions (NVC) during normal bladder filling. These NVC are responsible for incontinence episodes, bladder and bladder neck damage, as well as the life threatening consequences of hydronephrosis and autonomic dysreflexia. Our objectives and goals are to determine the relative contributions, if any, of the nervous system (parasympathetic, sympathetic and somatic) and intrinsic myogenic activity on the generation of high pressure NVC in chronic SCI rats with detrusor hyperreflexia and detrusor-sphincter dyssynergia. Once achieved, these insights may be translatable to novel therapeutic approaches toward alleviating detrusor hyperreflexia and detrusor-sphincter dyssynergia in humans with suprasacral spinal cord injury. Our overarching hypothesis is that NVCs in chronic SCI may be augmented by bladder-to-bladder parasympathetic reflexes, but are not themselves caused by bladder-to-bladder reflexes. Rather, two other sources are proposed: the first is myogenic filling contractions, which either invade the dome of the bladder causing a large amplitude myogenic contraction or cause a secondary reflex response to distension. This latter possibility is subtly yet importantly different than a response to gradual steady filling, as it implies an episodic distension of the dome which results in the spinal reflex. In either case, diminishing these myogenic contractions should reduce or eliminate the generation of large amplitude NVCs. The second possibility is one of indirect stimulation of the bladder by bladder-to-somatic reflexes, such that bladder filling, be it steady or episodic, evokes a limited mass-reflex of the abdominal musculature. This contraction of abdominal muscle then stimulates the bladder contraction by pressure generation via compression.

BODY: The approved tasks of the statement of work are as follows:

> **Task 1.** Development, submission and approval of an IACUC protocol covering the intended research (2 months)

Task 1 was accomplished and we proceeded to Task 2.

> **Task 2.** SA1: Determination of the autonomic neural and myogenic contribution to NVCs in conscious SCI rats. This task will take 18 rats/group in order to achieve 12 rats with detrusor hyperreflexia, detrusor-sphincter DSD and recovered voiding in order to achieve the aim's goals (75% of chronic SCI animals fall into this category, assume a loss of two animals/group due to morbidity/mortality associated with chronic SCI)
> **2a.** Ordering of animals, initial surgical preparation of SCI rats (1.25 months)
> **2b.** Weekly experiments with random assignments of animals to either treatments consistent with SA1a, SA1b, Sa1c or Control across a 14.5 month period (15. 5 months, includes 4 weeks of vacation/holiday time in addition to the 16.5 month experiment period)
> **2c.** Final data analysis (data analysis will be ongoing throughout, this will represent the finalization of data period, 0.25 months)
> **Total Time for Task 2** – 17 months
> **Total Time for Tasks 1 and 2** – 19 months

Task 2 was partially accomplished. We accomplished Sub-Tasks 2a and 2b. As of 9/29/13, we are in the process of data analysis, but have completed the measurements of the Control Data (repeated vehicle administration) and have analyzed bladder pressure parameters for this group. Other parameters from this group and all data from the other groups at the time need to be measured and/or analyzed.

Task 2b - During the course of the first year, we accomplished all of the Specific Aim 1 experiments. This includes the following Specific Aims:

Group	1st Treatment	2nd Treatment	3rd Treatment	4th Treatment
SA1a – Parasympathetic antagonists	Vehicle	Atropine (0.4 mg/kg)	NF-449 (10 mg/kg)	Hexamthonium (25 mg/kg)
SA1b – Sympathetic antagonists	Vehicle	Phentolamine (10 mg/kg)	Propranolol + SR 59230A (1 mg/kg / 1 mg/kg)	Hexamthonium (25 mg/kg)
SA1c – Smooth muscle blockers	Vehicle	Nifedipine (30 mg/kg)	CL-316243 (100 µg/kg)	Isoproternol (100 µg/kg)
Control	Vehicle	Vehicle	Vehicle	Vehicle

The data from the Control group only have been analyzed and demonstrate that repeated vehicle administration had no effect on True Bladder Capacity (TBC; estimated by single fill cystometrogram from empty bladder starting point), Functional Bladder Capacity (FBC; a.k.a. intermicturition interval during continuous cystometry, is affected by residual volumes due to decreased voiding efficiencies and is therefore not an estimate of TBC, but is what one can expect during normal function), or Maximum NVC Amplitude. However, there was a statistically significant increase (albeit not dramatic in physiological significance) in Voiding Efficiency (%VE; P=0.0129 by Friedman Test, $p<0.05$ for ~10% increase at 4th Vehicle dose. These data can be seen in Figure 1, and these results provide the necessary backdrop for appropriate 2-Way ANOVA analysis for the remaining groups. It is with some relief that we find that there are no physiologically significant effects of repeated vehicle administration, although our study was designed to account for this by inclusion of this very group.

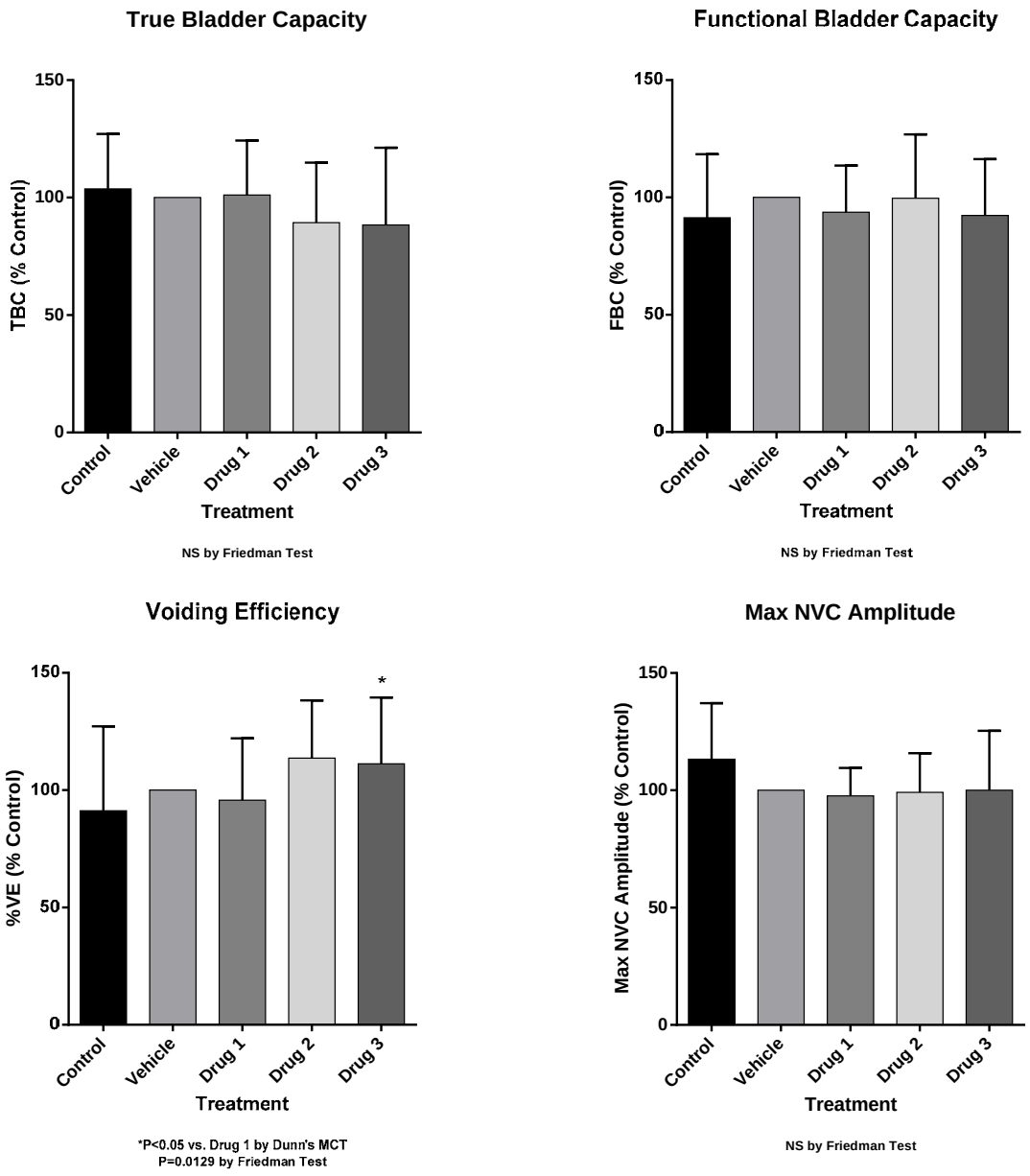

Figure 1 – Graphical representations of the repeated vehicle control data from Specific Aim 1. Upper left is True Bladder Capacity, upper right is Functional Bladder Capacity, lower left is Voiding Efficiency, lower right is Maximum NVC Amplitude. Data from each animal have been normalized to the first vehicle response. Drug 1 – 3 in this case refers to repeated vehicle injections. Note no dramatic effect of repeated vehicle on these parameters of neurogenic bladder function.

We also made some general observations, and perhaps the most key of the study thus far. These include that following Hexamethonium administration, all true voiding events (spinal micturition reflex, complete with phasic external urethral sphincter firing) are eliminated, however, voiding occurred by a combination of augmented overflow incontinence (NVC-driven and a vesicosomatic reflex of the hindquarters (Figure 2)

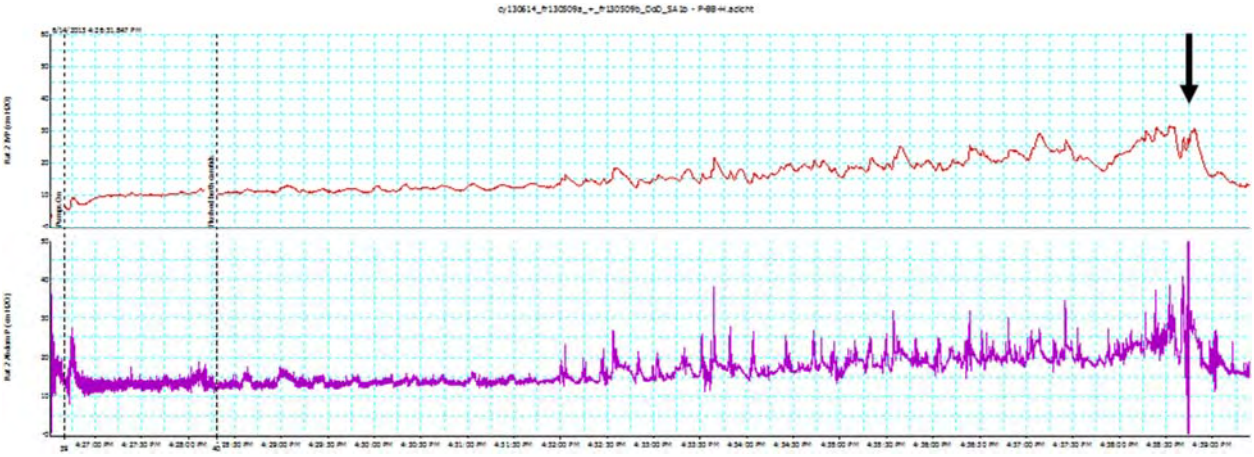

Figure 2 – Bladder pressure tracing (top panel) and intra-abdominal pressure tracing (bottom panel) following hexamethonium administration. Note that the two traces are quasi-superimposable, suggesting that either the bladder pressure drives the abdominal pressure, or that abdominal pressure is largely responsible for bladder activity under these conditions. Also note that the voiding contraction at the end (black arrow) is not a spinal micturition reflex, but rather an augmented overflow incontinence with a locomotor component (High amplitude pressure swings in bottom trace at time indicated by the black arrow).

Additionally, in all animals there was a highly suspicious relationship between intra-abdominal pressure and NVCs in the bladder (Figure 3), such that they are either superimposable OR it is clear that the abdominal pressure rise precedes the bladder pressure rise, the latter suggesting that abdominal pressure rise is itself a driving force for the bladder NVC.

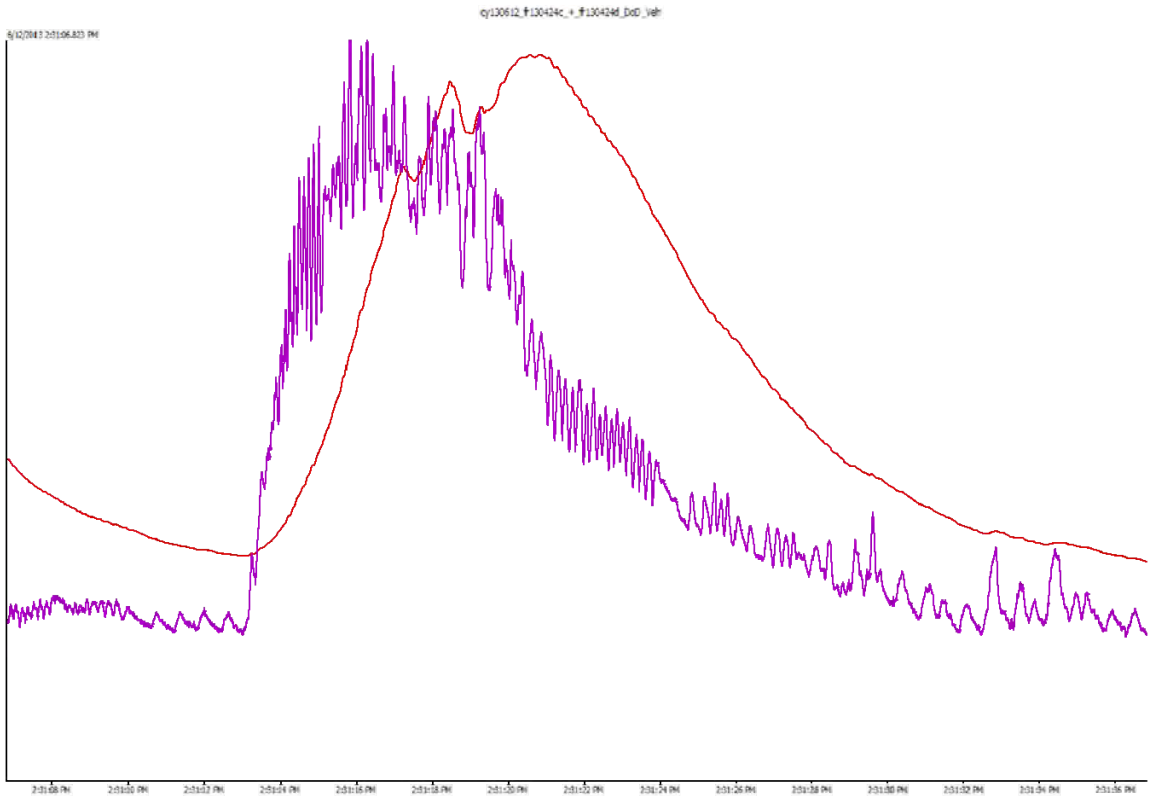

Figure 3 – Superimposed intra-abdominal pressure (purple trace) and bladder pressure (red trace) during an NVC. While the trace suggests that intra-abdominal drives the bladder, one could also argue that both occur at the same time as part of a mass reflex, and that the intra-abdominal pressure leads that of the bladder because of the kinetics of striated vs. smooth muscle. Specific Aim 2 will definitively answer this question.

We encountered some issues with reliable blood pressure recordings toward the end, but this was due to catheter tip placement and we have remedied this issue. We also had personnel issues, with one technician leaving abruptly and hiring and training a new one. The writing of this report has been delayed, in part, by our lab moving to a new building and maternity leave of the new technician putting many things behind as the PI had to perform many of the duties. All things are now in place and we look forward to a smoother future.

Task 3. <u>SA2: Determination of extra-vesicular contributions to NVCs in decerebrate chronic SCI rats.</u> A total of 20 rats will be required to achieve this aim's goal. 18 for the same reasons as given for each group size in Task 2, and an additional 2 rats for mortality/morbidity due to decerebration.
3a. Ordering of animals, initial surgical preparation of SCI rats (1.25 months, but happened during Task 2, so not counted within Task 3)
3b. Weekly experiments across a 4.5 month period.
3c. Data analysis and report writing – ongoing and finalized within 0.5 months.

Total Time for Task 3 – 5 months
Total Time for Entire Project – 24 months

As of 9/29/13, we have not begun Task 3.

KEY RESEARCH ACCOMPLISHMENTS:

- Testing of parasympatholytic agents on lower urinary tract function in chronic suprasacral spinal cord injured rats during conscious, restrained cystometry
- Testing of sympatholytic agents on lower urinary tract function in chronic suprasacral spinal cord injured rats during conscious, restrained cystometry
- Testing of smooth muscle relaxant agents on lower urinary tract function in chronic suprasacral spinal cord injured rats during conscious, restrained cystometry
- Testing of repeated vehicle administration on lower urinary tract function in chronic suprasacral spinal cord injured rats during conscious, restrained cystometry
- Observations of pressure-driven vesico-somatic reflexes resulting in both abdominal contraction and locomotor activity

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- No reportable outcomes as of the end date of this report, but preparation of abstracts for meetings will begin shortly, as soon as the data analyses allow.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

We have thus far demonstrated that repeated vehicle administration in our hands with our preparation does not result in physiologically significant changes in voiding parameters (with only voiding efficiency being statistically significantly increased by 10% at the fourth vehicle when compared to the second, importantly not the first). This portends well for the comparisons of our results to drug treatments, with analyses underway.

We have also observed that intra-abdominal pressure and intravesical pressure have an intimate relationship, suggesting one drives the other, and that may be different depending on the event (baseline vs. NVC). Together with Specific Aim 2, we are poised to determine the relationship between the bladder-to-bladder reflex and either a bladder-to-abdominal musculature-bladder reflex or a bladder-to-mass reflex which drives them both simultaneously.

We may also state that none of the drug treatments except for hexamethonium can eliminate the spinal micturition reflex, but this conclusion must be reviewed more carefully to ensure that voiding is not actually occurring by a bladder-to-hindlimb locomotor reflex, driven by high pressures.

These results are important, as they will elucidate the relationship(s) between the urinary bladder and the somatic motor system following suprasacral SCI. That abdominal pressure may be responsible for the high pressure NVC seen in this condition dramatically alters our approach in treating this dangerous phenomenon. Best approaches may include elimination of the triggers, likely to be myogenic base-to-dome filling contractions and/or central striated muscle relaxants.

REFERENCES:

None

APPENDICES:

None